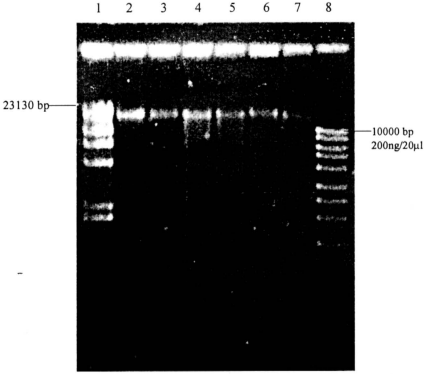
CHAPTER IV

RESULTS

4.1 DNA Yield

High concentration of urea (6M) containing buffer was able to lyse scales from young and adult Asian arowana. Scales which had been kept in various condition for periods ranging one week to two years yielded satisfactory quality genomic DNA (Fig. 1). The DNA yield was in the range of 0.8μg of total DNA/mg of preserved scale and 0.1μg of total DNA/mg of dried scale. The OD_{260}/OD_{280} ratios ranged from 1.84 to 2.0.

Fig. 4.1 Genomic DNA of Asian arowana obtained from scales using TNES-Urea buffer



Lane 1: Lambda DNA digested with λ*Hind III* (Promega)
Lane 2: DNA isolated from scale of young live fish
Lanes 3 to 6: DNA isolated from scales collected from fish dead from between 6 months to 2 years.
Lane 7: DNA extracted from a 6-month-old dried scale.
Lane 8: Mass Ruler^{TM} DNA ladder, high range (Fermentas)

4.2 Micorsatelite Variation

The phenotypes of each individual fish for every locus are listed in Appendix 4. Only specific phenotypes from each colour varieties were illustrated in Fig. 4.2a to Fig. 4.22.

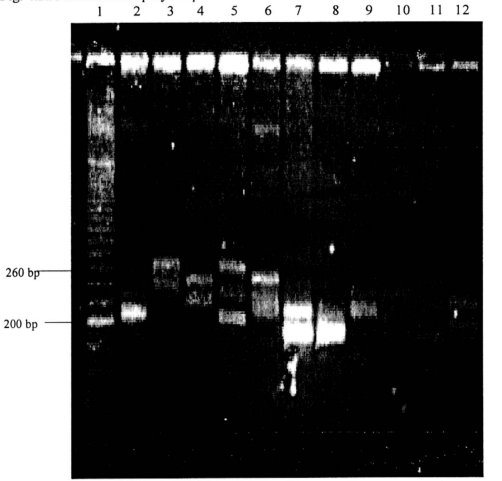
4.2.1 Locus *D01* (Fig. 4.2a and 4.2b)

Fig. 4.2a shows that the presence of five alleles in green arowana leading to four phenotypes. Only one of these phenotypes was homozygous. Four out of the five phenotypes observed were heterozygous (lanes 2 to 6). The Malaysian-red-tail gold individuals showed four phenotypes (lanes 7 to 12). There were PCR failures for Malaysian red-tail gold due to homozygosity for null alleles. Fig. 4.2b shows that five phenotypes were observed in Malaysian yellowtail gold (lanes 2 to 6). There were five phenotypes in red rowan but only one of them was homozygous (lanes 7 to 10). Two homozygous and a heterozygous phenotypes were found in Indonesian gold (lanes 11 to 14).

4.2.2 Locus *D04* (Fig. 4.3a and 4.3b)

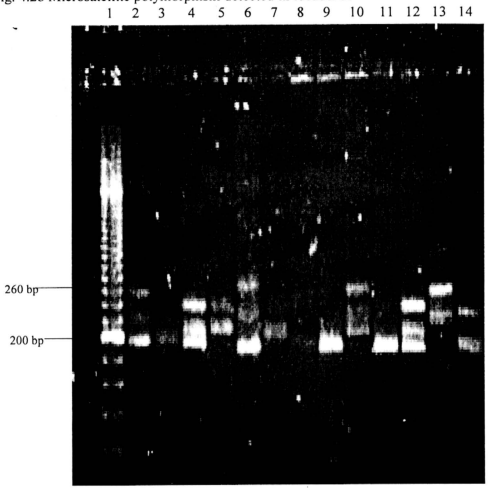
Three heterozygous phenotypes were found in green arowana (Fig. 4.3a, lanes 2 to 5). The Malaysian red-tail gold were more variable, with seven phenotypes observed. Out of the seven phenotypes, only one was homozygous (lanes 6 to 12). The microsatellite profiles of the Malaysian yellow-tail gold consisted of four patterns but only one was homozygous (lanes 13 to 17). In Fig. 4.3b, Indonesian gold also showed four different phenotypes (lanes 2 to 5). Red arowana were less variable, with three phenotypes detected (lanes 6 to 10).

Fig. 4.2a Microsatellite polymorphism detected for locus *D01*



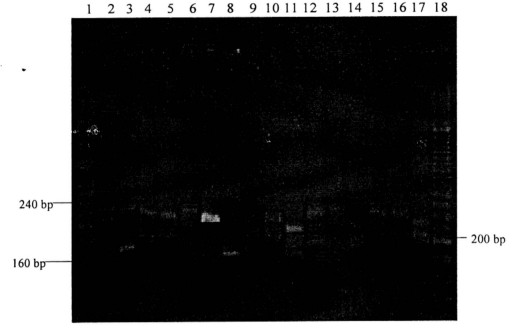
Lane 1: 20 bp ladder
Lanes 2 to 6 : Green arowana
Lanes 7 to 12 : Malaysian-red-tail gold

Fig. 4.2b Microsatellite polymorphism detected in locus *D01*



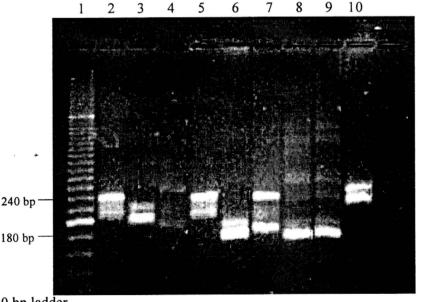
Lane 1: 20 bp: ladder
Lanes 2 to 6: Malaysian yellow-tail gold
Lanes 7 to 10: Indonesian gold arowana
Lanes 11 to 14: Red arowana

Fig. 4.3a Microsatellite polymorphisms detected in locus *D04*



Lane 1 and 18: 20 bp ladder
Lanes 2 to 5: Green arowana
Lanes 6 to 12: Malaysian-red-tail gold
Lanes 13 to 17: Malaysian yellow-tail gold

Fig. 4.3b Microsatellite polymorphisms detected in locus *D04*



Lane 1: 20 bp ladder
Lanes 2 to 5: Indonesian gold
Lanes 6 to 10: Red arowana

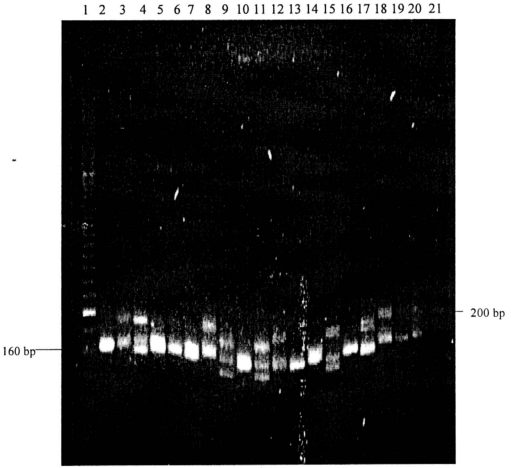
4.2.3 Locus *D11* (Fig. 4.4)

Three different phenotypes were observed in green arowana (lanes 2 to 5). These phenotypes were due to four alleles with different sizes. Locus *D11* in Malaysian red-tail gold showed four phenotypes due to three alleles (lanes 6 to 10). Four alleles contributed to the four phenotypes in the Malaysian yellow-tail gold (lanes 11 to 14). Four different phenotypes were found in the Indonesian gold coded for by the four alleles of this locus (lanes 15 to 17). Two alleles were observed in the red arowana and they resulted in two phenotypes (lanes 18 to 20).

4.2.4 Locus *D13* (Fig. 4.5)

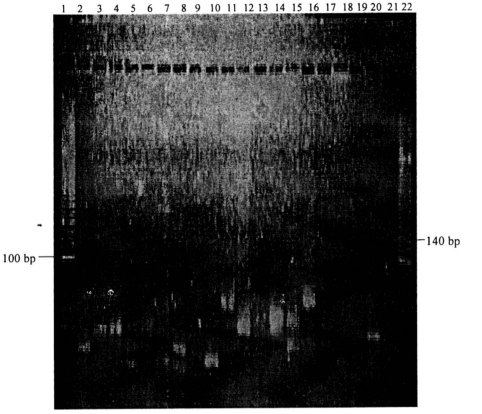
The green arowana individuals were monomorphic (lane 2). The microsatellite profiles of the Malaysian red-tail gold consisted of five patterns, two were homozygous and three were heterozygous (lanes 3 to 9). The Malaysian yellow-tail individuals showed homozygous phenotypes (lanes 10 to 12). Two homozygous and a heterozygous phenotypes resulted from the presence of three alleles in the Indonesian gold (lanes 13 to 17). Three alleles were found in red arowana but all the red individuals were homozygous (lanes 18 to 21).

Fig. 4.4 Microsatellite polymorphisms detected in locus *D11*



Lanes 1 and 21: 20 bp ladder
Lanes 2 to 5: Green arowana
Lanes 6 to 10: Malaysian-red-tail gold
Lanes 11 to 14: Malaysian yellow-tail gold
Lanes 15 to 17: Indonesian gold arowana
Lanes 18 to 20: Red arowana

Fig. 4.5 Microsatellite polymorphisms detected in locus *D13*

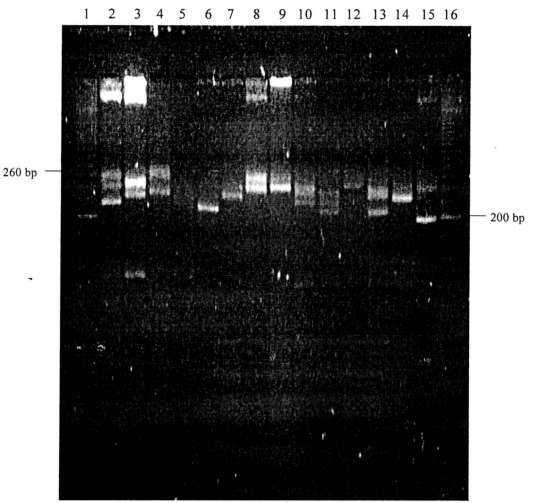


Lanes 1 and 22: 20 bp ladder
Lane 2: Green Arowana
Lanes 3 to 9: Malaysian-red-tail gold
Lanes 10 to 12: Malaysian yellow-tail gold
Lanes 13 to 17: Indonesian gold arowana
Lanes 18 to 21: Red arowana

4.2.5 Locus *D14* (Fig. 4.6)

There were three alleles in green arowana. These alleles led to two phenotypes (lanes 2 to 3). All the green individuals were homozygous. The Malaysian-red-tail gold individuals showed three phenotypes (lanes 4 to 6) while two phenotypes were observed in the Malaysian yellow-tail gold (lanes 7 and 8). Fig. 8 illustrates these four phenotypes found in red arowana (lanes 12 to 15) and the three found in the Indonesian gold (lanes 9 to 11).

Fig. 4.6 Microsatellite polymorphisms detected in locus *D14*



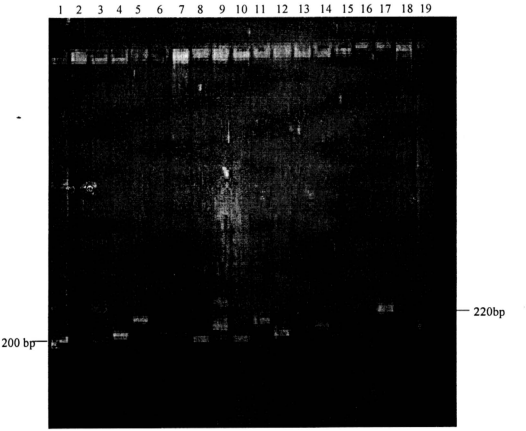
Lanes 1 and 16: 20 bp ladder
Lanes 2 to 3: Green arowana
Lanes 4 to 6: Malaysian-red-tail gold
Lanes 7 to 8: Malaysian yellow-tail gold
Lanes 9 to 11: Indonesian gold arowana
Lanes 12 to 15: Red arowana

4.2.6 Locus *D15* (Fig. 4.7)

Three alleles resulted in two phenotypes in green arowana (lanes 2 to 4). Four different banding patterns were observed in Malaysian red-tail gold (lanes 8 to 11). They were due to four alleles. On the other hand, two alleles in the Malaysian yellow-tail gold led to a homozygous and a heterozygous phenotypes (lanes 5 to 7). A null

allele, which led to PCR failure, was detected in the green, Malaysian red-tail gold, Malaysian yellow-tail gold and red arowana (not shown). Four alleles resulting in three phenotypes were observed in the Indonesian gold (lanes 12 to 15). Only one individual was heterozygous and the rest were homozygous. There were two alleles in the red arowana but all the red individuals were homozygous (lanes 17 to 19).

Fig. 4.7 Microsatellite polymorphisms detected in locus *D15*

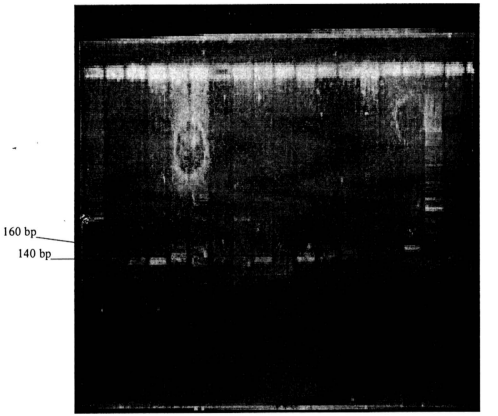


Lanes 1 and 19: 20 bp ladder
Lanes 3 to 4: Green arowana
Lanes 5 to 7: Malaysian yellow-tail gold
Lanes 8 to 11: Malaysian-red-tail gold
Lanes 12 to 15: Indonesian gold arowana
Lanes 17 to 19: Red arowana

4.2.7 Locus *D16* (Fig. 4.8)

Fig. 4.8 shows that the green, Malaysian red-tail gold and Malaysian yellow-tail gold arowana shared a homozygous and a heterozygous phenotypes (lanes 2 to 12). There were three phenotypes in the Indonesian gold. Only two individuals showed heterozygosity (lanes 14 and 15). Two alleles resulted in three phenotypes in the red arowana but only two individuals were heterozygous (lanes 16 to 18).

Fig. 4.8 Microsatellite polymorphisms detected in locus *D16*



Lanes 1 and 17: 20 bp ladder
Lanes 2 to 5: Green arowana
Lanes 6 to 9: Malaysian-red-tail gold
Lanes 10 to 12: Malaysian yellow-tail gold
Lanes 13 to 15: Indonesian gold arowana
Lanes 16 and 18: Red arowana

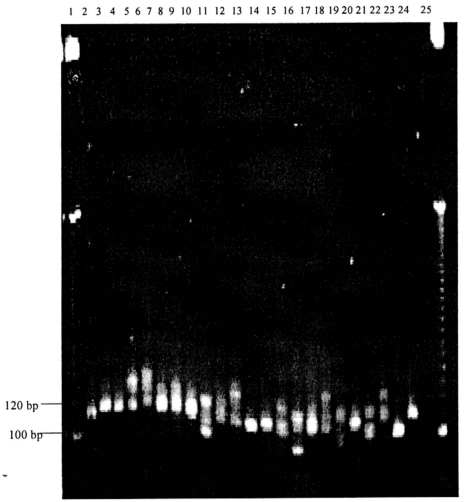
4.2.8 Locus *D27* (Fig. 4.9)

In the green arowana, five different amplification products, contributed by four alleles were observed (lanes 2 to 7). There were four types of banding patterns in the red arowana but these patterns were due to four alleles only (lanes 21 to 24). PCR failure occurred in green and red arowana and this could be caused by null alleles (not shown). Four alleles gave rise to four phenotypes in the Malaysian red-tail gold (lanes 8 to 12). Four phenotypes were found in the Malaysian yellow-tail gold and five alleles were observed (lanes 13 to 16). For the Indonesian gold, three alleles led to three phenotypes (lanes 17 to 20).

4.2.9 Locus *D31* (Fig. 4.10)

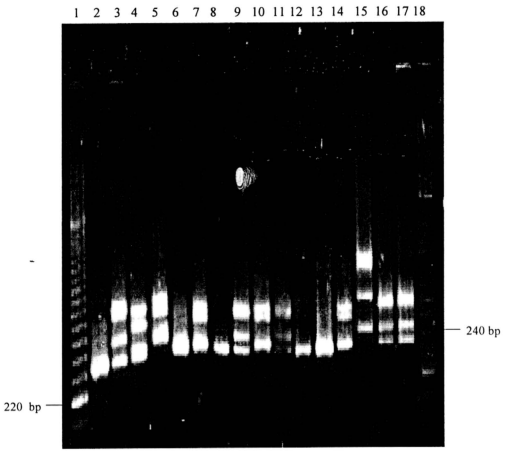
Three alleles gave rise to four phenotypes in the green arowana (lane 2 to 5). Three alleles resulted three phenotypes in the Malaysian red-tail gold but only one individual was heterozygous (lanes 6 to 9) and the same phenomenon was observed in the Malaysian yellow-tail gold (lanes 10 to 12). Two homozygous phenotypes were found in the Indonesian gold (lanes 13 and 14). Two alleles contributed to two phenotypes in the red arowana (lanes 15 to 17).

Fig. 4.9 Microsatellite polymorphisms detected in locus *D27*



Lanes 1 and 25: 10 bp ladder
Lanes 2 to 7: Green arowana
Lanes 8 to 12: Malaysian-red-tail gold
Lanes 13 to 16: Malaysian yellow-tail gold
Lanes 17 to 20: Indonesian gold arowana
Lanes 21 to 24: Red arowana

Fig. 4.10 Microsatellite polymorphisms detected in locus *D31*



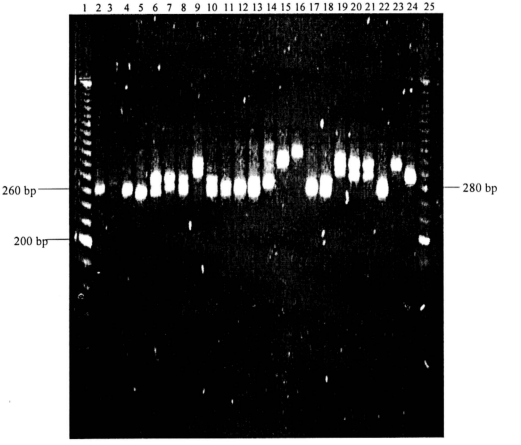
Lanes 1 and 18: 20 bp ladder
Lanes 2 to 5: Green arowana
Lanes 6 to 9: Malaysian-red-tail gold
Lanes 10 to 12: Malaysian yellow-tail gold
Lanes 13 to 14: Indonesian gold arowana
Lanes 15 to 17: Red arowana

4.2.10 Locus *D32* (Fig. 4.11)

Three alleles resulted in three different patterns in green arowana (lanes 2 to 7). For the Malaysian red-tail gold, five alleles were present for this locus and four banding patterns were detected (lanes 8 to 11). The microsatellite profiles showed five patterns due to the five alleles observed in the Malaysian yellow-tail gold (lane 12 to 16). Only

two alleles were present in the Indonesian gold, resulting in two homozygous phenotypes (lanes 17 and 18). For the red arowana, four banding patterns were caused by four alleles (lanes 19 to 24).

Fig. 4.11 Microsatellite polymorphisms detected in locus *D32*



Lanes 1 and 25: 20 bp ladder
Lanes 2 to 7: Green arowana
Lanes 8 to 11: Malaysian-red-tail gold
Lanes 12 to 16: Malaysian yellow-tail gold
Lanes 17 to 18: Indonesian gold arowana
Lanes 19 to 24: Red arowana

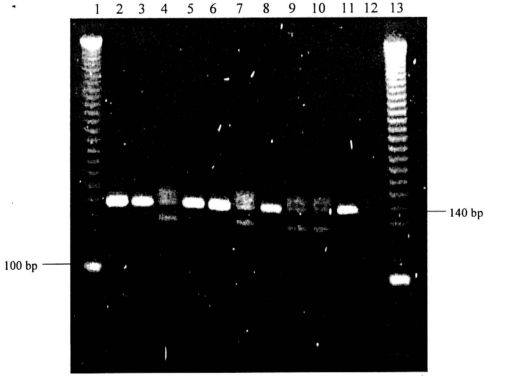
4.2.11 Locus *D33* (Fig. 4.12)

Only two patterns of amplification products were obtained for this locus. This locus was monomorphic in green arowana but variations were observed in the other strains.

4.2.12 Locus *D35* (Fig. 4.13)

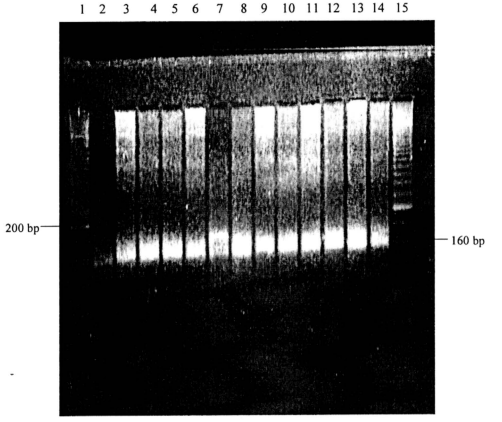
All the individuals shared a common allele. No variation was detected in green, Malaysian yellow-tail, Indonesian gold and red arowana. Malaysian red-tail gold showed polymorphisms with two alleles (lanes 6 and 7).

Fig. 4.12 Microsatellite polymorphism detected in locus D33



Lanes 1 and 13: 10 bp ladder
Lanes 2 to 3: Green arowana
Lanes 4 to 5: Malaysian-red-tail gold
Lanes 6 to 7: Malaysian yellow-tail gold
Lanes 8 to 9: Indonesian gold arowana
Lanes 10 to 12: Red arowana

Fig. 4.13 Microsatellite polymorphisms detected in locus *D35*

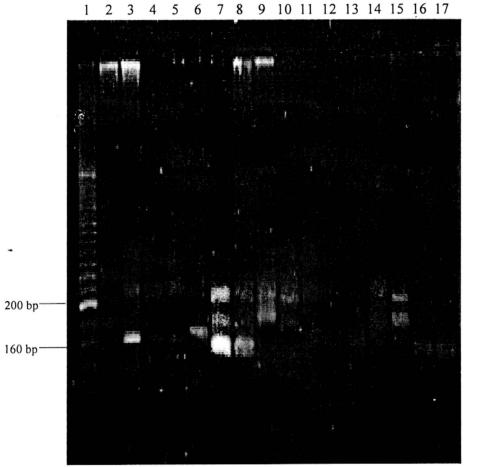


Lanes 1 and 15: 20 bp ladder
Lanes 2 to 3: Green arowana
Lanes 4 to 6: Malaysian yellow-tail gold
Lanes 7 to 8: Malaysian-red-tail gold
Lanes 9 to 12: Indonesian gold
Lanes 13 to 14: Red arowana

4.2.13 Locus *D37* (Fig. 4.14a and 4.14b)

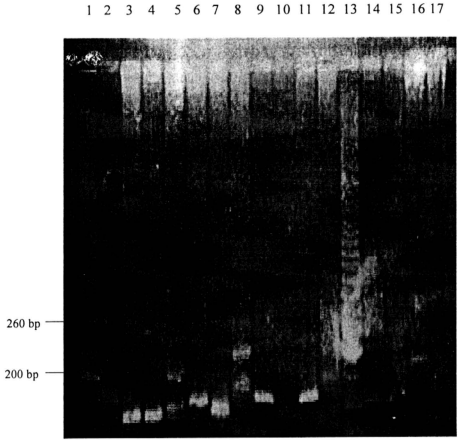
Fig. 4.14a shows that the phenotypes of the Malaysian red-tail gold were highly variable. There were five heterozygous and two homozygous phenotypes, due to five alleles (lanes 2 to 10). Three out of the four phenotypes in green arowana were homozygous (Fig. 4.14b, lanes 2 to 7). Three phenotypes were found in red, Malaysian yellow-tail gold and Indonesian gold arowana but the phenotypes of each strain were due to different alleles.

Fig. 4.14a Microsatellite polymorphisms detected in locus *D37*



Lanes 1: 20 bp ladder
Lanes 2 to 10: Malaysian-red-tail gold
Lanes 11 to 17: Malaysian yellow-tail gold

Fig. 4.14b Microsatellite polymorphisms detected in locus *D37*



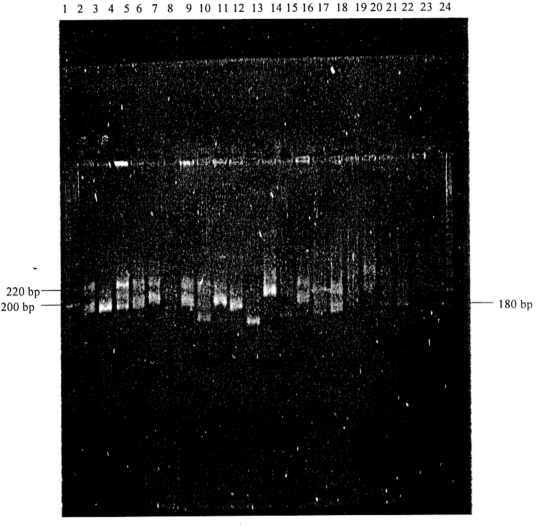
Lanes 1 and 13: 20 bp ladder
Lanes 2 to 7: Green arowana
Lanes 8 to 12: Indonesian gold arowana
Lanes 14 to 17: Red arowana

4.2.14 *Locus D38* (Fig. 4.15)

Most of the phenotypes detected were heterozygous. The green arowana showed two phenotypes (lanes 2 and 3). In the Malaysian red-tail gold, six alleles resulted in five phenotypes (lanes 4 to 9). Four phenotypes due to five alleles were found in the Malaysian yellow-tail gold (lanes 10 to 12). Null allele was detected in this strain (not shown). Four alleles led to five phenotypes in the Indonesian gold (lanes 13 to 16).

Only two phenotypes due to three alleles were observed in the red arowana (lanes 17 to 19).

Fig. 4.15 Microsatellite polymorphisms detected in locus *D38*



Lanes 1 and 24: 20 bp ladder
Lanes 2 to 5: Green arowana
Lanes 6 to 10: Malaysian yellow-tail gold
Lanes 11 to 16: Malaysian red-tail gold
Lanes 17 to 21: Indonesian gold arowana
Lanes 22 to 23: Red arowana

4.2.15 Locus *D42* (Fig. 4.16)

Three alleles were present in green arowana. This strain showed four phenotypes and only one is homozygous (lanes 2 to 5). There were only two alleles in the Malaysian red-tail gold and all the individuals were homozygous for this locus (lanes 6 to 8). Three alleles resulted in two heterozygous and a homozygous phenotypes in the Malaysian yellow-tail gold (lanes 9 to 12). There were three alleles in the Indonesian gold too, but only two phenotypes were observed (lanes 13 to 15). Four phenotypes due to four alleles were observed in the red arowana (lanes 16 to 19).

4.2.16 Locus *D72* (Fig. 4.17)

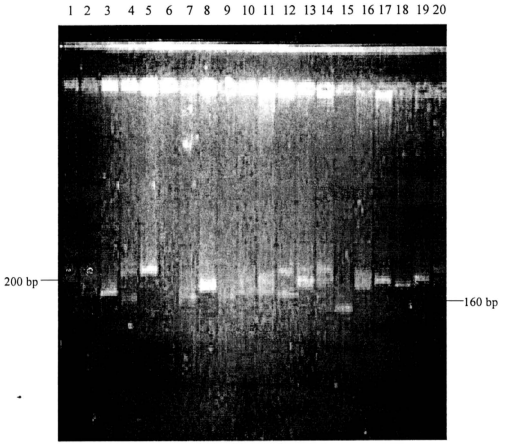
The allelic diversity of this locus was low. Only two alleles were detected in every strain of arowana. These alleles led to a homozygous and a heterozygous phenotypes in the green arowana (lanes 2 and 3). Two homozygous phenotypes were observed in the Malaysian red-tail gold (lanes 9 and 10) and the Indonesian gold (lanes 11 to 13) while three phenotypes were observed in the red (lanes 14 to 16) and Malaysian yellow-tail gold (lanes 4 to 8).

4.2.17 Locus *D85* (Fig. 4.18)

Three alleles contributed to four different patterns of amplification products in the green arowana (lanes 2 to 5). The Malaysian red-tail gold showed polymorphisms with four alleles and four phenotypes (lanes 6 to 11). Out of the four phenotypes observed, only one was heterozygous. Only two alleles with two different phenotypes were observed in the Malaysian yellow-tail gold (lanes 12 to 15). Two different amplification products due to three alleles were detected in the Indonesian gold (lanes 16 to 18). Three alleles with three banding patterns were found in the red arowana.

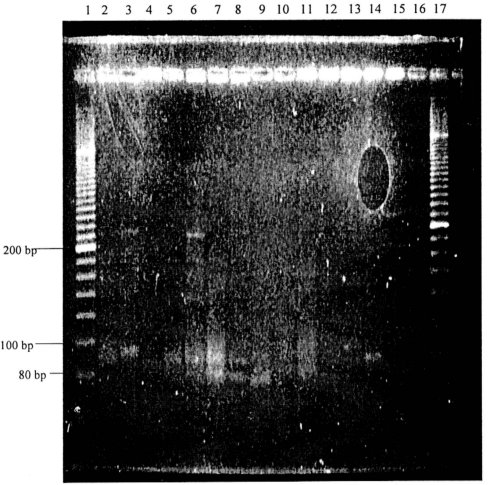
Two out of the three phenotypes in the red individuals were homozygous (lanes 19 to 21).

Fig. 4.16 Microsatellite polymorphisms detected in locus *D42*



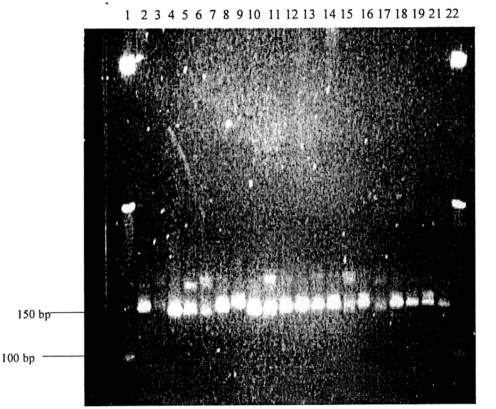
Lanes 1 and 20: 20 bp ladder
Lanes 2 to 5: Green arowana
Lanes 6 to 8: Malaysian-red-tail gold
Lanes 9 to 12: Malaysian yellow-tail gold
Lanes 13 to 15: Indonesian gold arowana
Lanes 16 to 19: Red arowana

Fig. 4.17 Microsatellite polymorphisms detected in locus *D72*



Langes 1 and 17: 20 bp ladder
Lanes 2 to 3: Green arowana
Lanes 4 to 8: Malaysian yellow-tail gold
Lanes 9 to 10: Malaysian-red-tail gold
Lanes 11 to 13: Indonesian gold
Lanes 14 to 16: Red arowana

Fig. 4.18 Microsatellite polymorphisms detected in locus *D85*



Lanes 1 and 22: 10 bp ladder
Lanes 2 to 5: Green arowana
Lanes 6 to 11: Malaysian-red-tail gold
Lanes 12 to 15: Malaysian yellow-tail gold
Lanes 16 to 18: Indonesian gold arowana
Lanes 19 to 21: Red arowana

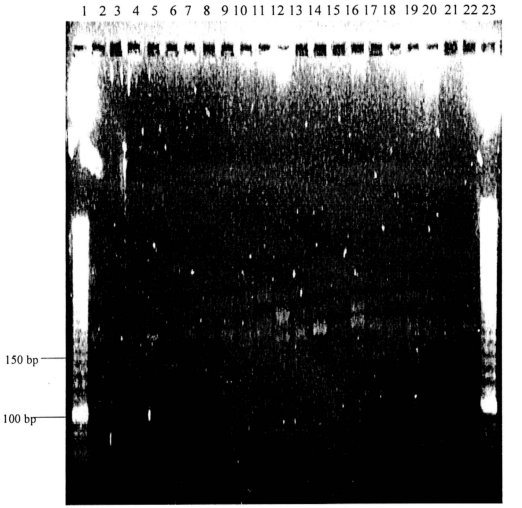
4.2.18 Locus *D88* (Fig. 4.19)

Five alleles were observed at this locus. The three alleles found in the green arowana contributed to three different phenotypes and one of them was homozygous (lanes 2 to 8). Four banding patterns were observed in the Malaysian red-tail gold (lanes 9 to 13) due to five alleles. Three out of the four phenotypes observed were heterozygous. Only two alleles were present in the Malaysian yellow-tail gold (lanes 14 to 16) and the red arowana (lanes 20 to 22). These two colour strains shared the same two banding patterns. Three alleles were detected in the Indonesian gold and three different patterns were observed (lanes 17 to19). Null allele was detected in the Malaysian red-tail gold and the red arowana (not shown).

4.2.19 Locus *D92* (Fig. 4.20)

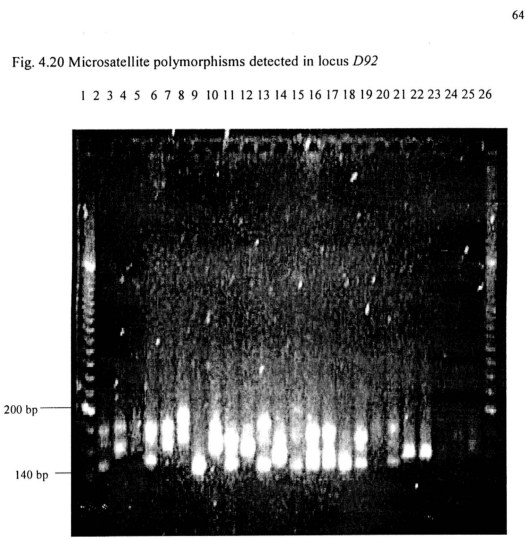
Five alleles were present in the green arowana. These resulted in three phenotypes (lanes 2 to 7). All the green individuals are heterozygous. There were seven different phenotypes due to six alleles in the Malaysian red-tail gold (lanes 5 to 12). Two were homozygous and the rest were heterozygous. Three heterozygous and a homozygous phenotypes were found in the red arowana (lanes 21 to 25 due to four alleles. There were four alleles in the Malaysian yellow-tail gold too but these alleles only gave rise to three phenotypes (lanes 13 to 16). Six alleles were detected in the Indonesian gold. Out of five phenotypes detected, only one was homozygous (lanes 17 to 20).

Fig. 4.19 Microsatellite polymorphisms detected in locus *D88*



Lanes 1 and 23: 10 bp ladder
Lanes 2 to 8: Green arowana
Lanes 9 to 13: Malaysian-red-tail gold
Lanes 14 to 16: Malaysian yellow-tail gold
Lanes 17 to 19: Indonesian gold arowana
Lanes 20 to 22: Red arowana

Fig. 4.20 Microsatellite polymorphisms detected in locus *D92*



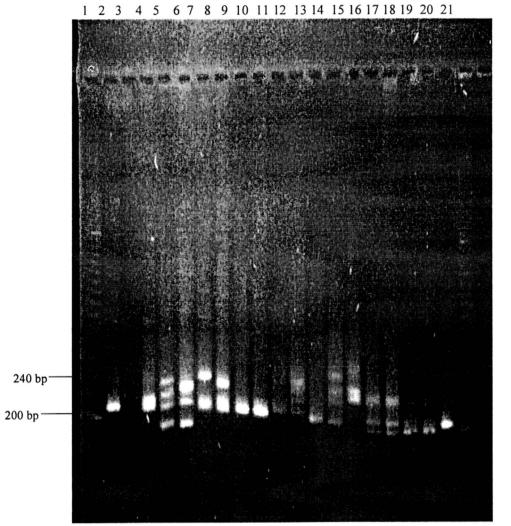
Lanes 1 and 26: 20 bp ladder
Lanes 2 to 5: Green arowana
Lanes 6 to 12: Malaysian-red-tail gold
Lanes 13 to 15: Malaysian yellow-tail gold
Lanes 16 to 20: Indonesian gold arowana
Lanes 21 to 25: Red arowana

4.2.20 Locus *D94* (Fig. 4.21)

Four phenotypes appeared in the green arowana (lanes 2 to 7). Only one of them was homozygous. These phenotypes were due to four alleles. Only two phenotypes with two alleles were detected in the Malaysian red-tail gold (lanes 8 and 9). Two alleles with three phenotypes existed in the red (lanes 16 to 20) whereas three alleles only

contributed to two phenotypes in the Malaysian yellow-tail gold (lanes 10 and 11). There were three alleles in the Indonesian gold, resulting in three phenotypes (lanes 12 to 15).

Fig. 4.21 Microsatellite polymorphisms detected in locus *D94*

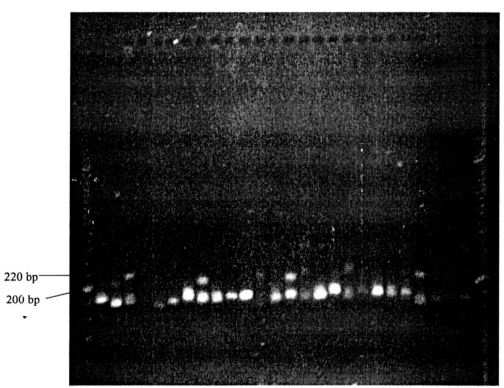


Lanes 1 and 21: 20 bp ladder
Lanes 2, 4 to 7: Green arowana
Lanes 8 to 9: Malaysian-red-tail gold
Lanes 10 to 11: Malaysian yellow-tail gold
Lanes 12 to 15: Indonesian gold arowana
Lanes 16 to 20: Red arowana

4.2.21 ocus *D95* (Fig. 4.22)

For the green arowana, there were three alleles at this locus, that gave rise to four phenotypes (lanes 2 to 6). Three alleles, with four phenotypes were observed in the Malaysian red-tail gold (lanes 7 to 11). The red (lanes 21 to 27) and the Malaysian yellow-tail gold (lanes 11 to 14) shared two phenotypes but differed in another two. Four phenotypes due to four alleles were found in the Indonesian gold (lanes 15 to 20).

Fig. 4.22 Microsatellite polymorphisms detected in locus *D95*

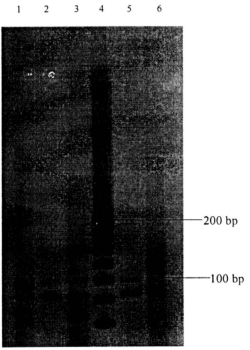


Lanes 1 and 28: 20 bp ladder
Lanes 2 to 6: Green arowana
Lanes 7 to 11: Malaysian-red-tail gold
Lanes 11 to 14: Malaysian yellow-tail gold
Lanes 15 to 20: Indonesian gold arowana
Lanes 21 to 27: Red arowana

4.3Comparison between Different Laboratory Procedures

Samples with different fragment sizes were chosen and run on polyacrylamide gels to compare the effects of different laboratory procedures. Fig. 4.23 and Table 4.1 shows that there was a few bp difference between the two techniques.

Fig. 4.23 Microsatellite polymorphisms detected in locus *D27* on polyacrylamide gel



Lanes 1 and 6: Green arowana
Lanes 2, 3 and 5: Malaysian yellow-tail gold
Lane 4: 20 bp ladder

Table 4.1. Different fragment sizes on MetaPhor agarose and polyacrylamide gels

Locus	Alleles	
	MetaPhor agarose gels	Polyacrylamide gels
D11	150	135
	155	145
	160	150
	165	155
	190	170
D27	95	85
	100	95
	115	100
	120	115
	125	120
	130	125
	135	130
D32	250	250
	260	260
	270	270
	280	280
	290	290
	300	300
	320	320
	330	330
D85	140	140
	145	150
	150	155
	160	160
D92	150	145
	155	150
	160	155
	170	165
	180	175
	190	185
	200	195
D95	170	150
	175	160
	180	170
	205	180

4.4 Genetic Variability among Loci

All the microsatellite loci isolated from green arowana were detected in the gold and the red arowana. Table 4.2 shows that a total of 109 alleles were observed from the 21 loci over 7 strains (91 individuals). Overall, genetic variability ranged widely among the loci. The observed number of alleles ranged from a low of two alleles to a high of eight alleles at each locus. Among the 21 loci analyzed, the highest degree of polymorphism was found at locus *D32*, with total eight alleles detected. Seven alleles each were detected at four loci (*D32*, *D37*, *D38* and *D92*). Only two alleles each were observed at loci *D33*, *D35* and *D72*. Locus *D35* was the least polymorphic one, showing only two alleles in the Malaysian red tail-gold arowana and was monomorphic in the other strains.

4.5 Genetic Diversity Among Strains

The allelic frequency distributions for two wild and five reared strains at each locus are listed in Table 4.2. The results showed that the numbers of polymorphic loci for the two wild strains (10 to 13 loci) were less than for the five reared strains (13 to 18 loci). The wild green arowana strain was found to have the lowest genetic diversity. This strain had only ten polymorphic microsatellite loci out of twenty-one loci analyzed. On the other hand, the other strains showed higher numbers of polymorphic loci and average genetic diversity values. Among these strains, the Indonesian gold arowana had the highest number of polymorphic loci followed by the Malaysian red-tail gold arowana. The results indicated that the 7 strains of arowana were not equally diverse.

The average observed allele number at individual loci ranged from 1.71 (wild green arowana) to 3.71 (Malaysian red-tail gold arowana). The highest allele number was found in the Malaysian red-tail gold arowana when compared with the other strains. However, more samples were collected from this strain. The allele numbers of the wild green arowana and the wild Malaysian yellow-tail gold arowana were low due to small sample size. The allele number in the Indonesian gold was strikingly high although sampling was neither extensive nor intensive. Thus, the average gene diversity over loci of the Indonesian gold arowana was the highest among the seven strains analyzed.

Null alleles were detected frequently in the Malaysian red-tail gold arowana especially at loci *D01*, *D11* and D15. For locus *D01*, three in 22 individuals showed PCR failure. Loci *D11* and *D15* had 7 and 6 individuals respectively for which no amplified product was observed (note that these individuals were different). Null alleles were also detected in other strains, but not as frequently as in the Malaysian red-tail gold arowana.

All of the strains shared twelve common alleles. These alleles were $D01^{210}$ (locus *D01*, allele size 210bp), $D11^{160}$, $D14^{220}$, $D16^{140}$, $D32^{250}$, $D33^{140}$, $D35^{160}$, $D37^{160}$, $D72^{90}$, $D88^{150}$, $D92^{190}$, $D95^{180}$. The frequencies of these common alleles were variable. Many differences between strains were due to unique alleles. A rare allele $D01^{260}$ with low frequency was only detected in the reared green arowana. Alleles $D32^{290}$, $D38^{170}$, $D88^{155}$, $D35^{180}$ were only detected in the Malaysian red-tail gold arowana. For the reared Malaysian yellow-tail gold arowana, three unique alleles ($D27^{95}$, $D27^{135}$ and $D32^{330}$) could differentiate this strain from the others. There were

Table 4.2: Allelic frequency distributions for twenty-one microsatellite loci in seven strains of arowana

Alleles (bp)	Wild Green	Farm Green	Malaysian Red-tail Gold	Red	Wild Malaysian Yellow-tail Gold	Farm Malaysian Yellow-tail Gold	Indonesian Gold
D01							
195			0.5000	0.5667	0.4167	0.5000	0.2727
210	0.9167	0.3571	0.3684	0.2000	0.2500	0.0938	0.4545
220		0.1786	0.1316	0.1000	0.2500	0.3438	
230	0.0833	0.2500		0.0333	0.0833	0.0625	
240		0.1786		0.1000			0.2727
260		0.0357					
No. of alleles	2	5	3	5	4	4	3
N	6	14	19	15	6	16	11
D04							
170		0.1154	0.0455				
180		0.1538	0.0909	0.8667			
190			0.2727	0.0333	0.3571	0.2812	0.2727
210	0.5000	0.1538	0.0909		0.4286	0.3438	0.0909
230	0.5000	0.5769	0.4545		0.0714	0.0938	0.2727
240			0.0455	0.0667	0.1429	0.2812	0.3636
250				0.0333			0.0455
No. of alleles	2	4	6	4	4	4	5
N	6	13	22	15	7	16	11

(continued)

(continued) Table 4.2: Allelic frequency distributions for twenty-one microsatellite loci in seven strains of arowana

D11							
150			0.0333			0.1875	0.1818
155		0.1429					0.1818
160	1.0000	0.3929	0.5667	0.8000	1.0000	0.7188	0.4546
165		0.0357	0.3333			0.0312	
190		0.4286	0.0667	0.2000		0.0625	0.0909
No. of alleles	1	4	4	2	1	4	4
N	6	14	15	15	7	16	11
D13							
95			0.0952	0.2000			
100			0.1667		0.1429	0.6875	
105	1.0000	1.0000	0.3810	0.2000			0.1364
120			0.3571	0.6000	0.8571	0.2500	0.6818
140						0.0625	0.1818
No. of alleles	1	1	4	3	2	3	3
N	6	14	21	15	7	16	11
D14							
200				0.0667			
210				0.1000			0.0909
215			0.5454				0.2727
220	0.5000	0.3929	0.1818	0.0667	0.4286	0.3750	0.0455
240		0.1071	0.1364	0.7000	0.2857	0.3125	0.5909
250	0.5000	0.5000		0.0333	0.2857	0.3125	
260			0.1364	0.0333			
No. of alleles	2	3	4	6	3	3	4
N	6	14	22	15	7	16	11

(continued) Table 4.2: Allelic frequency distributions for twenty-one microsatellite loci in seven strains of arowana

D15							
195	0.5000	0.1667	0.5938				0.3636
200		0.6667			0.2143	0.4333	0.5455
210	0.5000	0.1667	0.0625	0.2857			0.0455
220			0.2812	0.7143	0.7857	0.5667	
235			0.0625				0.0455
No. of alleles	2	3	4	2	2	2	4
N	5	12	16	14	7	15	11
D16							
130				0.5000			
140	1.0000	0.8214	0.8000	0.5000	0.7143	0.8214	0.7272
150		0.1786	0.2000		0.2857	0.1786	0.1818
160							0.0455
No. of alleles	1	2	2	2	2	2	3
N	6	14	22	15	7	14	11
D27							
95						0.6312	
100			0.0227	0.5000			0.0909
115	1.0000	0.6786	0.8863		0.7143	0.6562	0.7727
120		0.1786		0.2083		0.1875	
125			0.0455	0.2500		0.0312	0.1364
130		0.1071	0.0455	0.0417	0.2857	0.0938	
135		0.0357					
No. of alleles	1	4	4	4	2	5	3
N	6	14	22	12	7	16	11

(continued)

(continued) Table 4.2: Allelic frequency distributions for twenty-one microsatellite loci in seven strains of arowana

D31							
220	0.8333	0.4231	0.5925		0.1429	0.5312	0.8000
225		0.1154	0.3810	0.1333	0.8571	0.4375	0.2000
240	0.1667	0.4615	0.0238	0.8667		0.0312	
No. of alleles	2	3	3	2	2	3	2
N	6	13	21	14	7	16	10
D32							
250	0.3333	0.3571	0.0455	0.0667	0.0714	0.0938	0.9545
260	0.3333	0.3214	0.8636		0.8571	0.5000	
270			0.0455		0.0714	0.2812	0.0455
280	0.3333	0.3214		0.2000			
290			0.0227				
300			0.0227	0.1333			
320				0.6000		0.0625	
330						0.0625	
No. of alleles	3	3	5	4	3	5	2
N	6	14	22	15	7	16	11
D33							
130			0.0227	0.1429		0.0312	0.0909
140	1.0000	1.0000	0.9773	0.8571	1.0000	0.9688	0.9091
No. of alleles	1	1	2	2	1	2	2
N	6	14	22	14	7	16	11

(continued)

(continued) Table 4.2: Allelic frequency distributions for twenty-one microsatellite loci in seven strains of arowana

D35							
160	1.0000	1.0000	0.9318	1.0000	1.0000	1.0000	1.0000
180			0.0682				
No. of alleles	1	1	2	1	1	1	1
N	6	14	22	15	7	16	11
D37							
160	0.6667	0.6071	0.2250	0.1333	0.4286	0.5938	0.1818
170		0.1429	0.4000				0.1818
180	0.3333	0.1429	0.1250		0.5000	0.2500	0.3182
200		0.1071	0.0500	0.1333			
210			0.2000		0.0714	0.1562	0.3182
220				0.3667			
240				0.3667			
No. of alleles	2	4	5	4	3	3	4
N	6	14	20	15	7	16	11
D38							
170			0.0455				
180			0.2045	0.3333	0.0714	0.1000	0.2727
190			0.2500		0.0714	0.1333	
200	0.6667	0.6786	0.1818		0.2857	0.4000	0.2273
220			0.1364	0.3333	0.2857	0.2000	0.1364
230	0.3333	0.3214	0.1818		0.2857	0.1667	
240				0.3333			0.3636
No. of alleles	2	2	6	3	5	5	4
N	6	14	21	15	7	15	11

(continued)

(continued) Table 4.2: Allelic frequency distributions for twenty-one microsatellite loci in seven strains of arowana

D42							
150							0.3636
160	0.1667	0.0769	0.0476		0.5714	0.6000	
180	0.3333	0.6154	0.9524	0.8333			
190				0.1000	0.1429	0.2000	0.3182
200				0.0333			
220	0.5000	0.3077		0.0333	0.2857	0.2000	0.3182
No. of alleles	3	3	2	4	3	3	3
N	6	13	21	15	7	15	11
D72							
90	1.0000	0.8214	0.1429	0.2500	0.0714	0.1000	0.0909
100		0.1786	0.8571	0.7500	0.9286	0.9000	0.9091
No. of alleles	1	2	2	2	2	2	2
N	6	14	21	14	7	15	11
D85							
140		0.3929	0.6591	0.3077	0.8571	0.9062	0.2727
145	1.0000	0.3929	0.0455	0.5769			0.4545
150			0.0455	0.1154			
160		0.2143	0.2500		0.1429	0.0938	0.2727
No. of alleles	1	3	4	3	2	2	3
N	6	14	22	13	7	16	11

(continued)

(continued) Table 4.2: Allelic frequency distributions for twenty-one microsatellite loci in seven strains of arowana

D88							
130		0.2500	0.0714				0.0909
150	1.0000	0.6786	0.7381	0.8077	0.8571	0.9062	0.6818
155			0.0476				
165		0.0714	0.0952	0.1923	0.1429	0.0938	0.2273
180			0.0476				
No. of alleles	1	3	5	2	2	2	3
N	6	14	22	13	7	16	11
D92							
150	0.3333	0.0357	0.2273		0.0714	0.0312	0.4500
155		0.0714		0.0667	0.1429	0.0938	0.1500
160			0.0227	0.7333	0.7143	0.8438	0.1000
170	0.1667	0.3929	0.4091				0.1000
180			0.2045	0.0333			0.0500
190	0.3333	0.1071	0.1136	0.1667	0.0714	0.0312	0.1500
200	0.1667	0.3929	0.0227				
No. of alleles	4	5	6	4	4	4	6
N	6	14	22	15	7	16	10

(continued)

(continued) Table 4.2: Allelic frequency distributions for twenty-one microsatellite loci in seven strains of arowana

D94							
205		0.1071					
210	1.0000	0.5714	0.6818	0.5333	1.0000	0.6250	0.2773
220		0.2500		0.4667		0.1875	0.0909
230		0.0714	0.3182				0.0455
240						0.1875	0.1364
No. of alleles	1	4	2	2	1	3	4
N	6	14	22	15	7	16	11
D95							
170				0.0333			
175		0.0833		0.2333	0.2143	0.3214	0.5000
180	0.5833	0.5417	0.5682	0.7000	0.7143	0.6429	0.3182
185	0.4167	0.3750	0.4091	0.0333			0.0455
205			0.0227		0.0714	0.0357	0.1364
No. of alleles	2	3	3	4	3	4	4
N	6	12	22	15	7	14	11
No. of polymorphic loci	10	13	16	13	16	14	18
Average gene diversity over loci	0.2759 ± 0.1616	0.4921 ± 0.2641	0.4301 ± 0.2290	0.4488 ± 0.2438	0.3560 ± 0.1995	0.4392 ± 0.2370	0.4992 ± 0.2657

four alleles (*D16*^{130}, *D37*^{240}, *D42*^{200} and *D95*^{170}), which could differentiate the red arowana from the other strains. Alleles *D16*^{160} and *D42*^{150} were only observed in the Indonesian gold arowana. Allele *D01*^{195} was absent from the wild and reared green arowana but occurred in the other five strains with high frequencies while allele *D15*^{235} was only detected in the Malaysian red-tail gold arowana and the Indonesian gold arowana with low frequenies. Allele *D14*^{260} was found in the Malaysian red-tail gold arowana and the red arowana. Most of these unique alleles had larger fragment sizes than the common alleles.

4.6 Hardy-Weinberg Equilibrium

The polymorphic loci were tested for deviations from Hardy-Weinberg equilibrium. A significant ($P < 0.05$) deficit of heterozygosity was observed at locus *D37* for the wild green arowana while the other nine loci were in Hardy-Weinberg equilibrium. However there was no departure from Hardy-Weinberg equilibrium after sequential Bonferroni correction.

Fourteen loci showed agreement with Hardy-Weinberg equilibrium in the farm bred green arowana (Table 4.3). Four loci (*D27*, *D42*, *D37* and *D95*) showed significant ($P < 0.05$) disagreement with Hardy-Weinberg equilibrium, showing significant deficiency of heterozygosity. After sequential Bonferroni correction, only locus D37 showed a highly significant heterozygosity deficit ($P < 0.01$).

For the Malaysian red-tail gold arowana, there were nine significant deviations from Hardy-Weinberg equilibrium (Table 4.3). Significant ($P < 0.05$) deficits of heterozygosity were observed at loci *D14*, *D15*, *D31*, *D32*, *D72*, *D85* and *D95*. After

sequential Bonferroni adjustment, two loci (*D31* and *D72*) showed strong significance ($P < 0.01$) of heterozygosity deficiencies while locus *D85* showed a weaker significance ($P < 0.05$) of heterozygosity deficiency.

For the red arowana, eleven significant deviations from Hardy-Weinberg equilibrium were observed (Table 4.3). These eleven significant ($P < 0.05$) deviations were at loci *D04*, *D13*, *D14*, *D15*, *D16*, *D32*, *D42*, *D72*, *D85* and *D95* which showed substantial heterozygosity deficiencies. On the other hand, significant ($P < 0.05$) heterozygote excess was observed at *D37*. Only four loci showed departure form Hardy-Weinberg equilibrium after sequential Bonferroni correction. Loci *D13*, *D15* and *D16* showed highly significant ($P < 0.01$) heterozygosity deficiencies while locus *D95* showed weak significance ($P < 0.05$).

In the wild Malaysian yellow-tail gold arowana, all the polymorphic loci except locus D95 were in Hardy-Weinberg equilibrium. Locus *D95* showed significant ($P < 0.05$) heterozygosity deficiencies. However, after sequential Bonferroni correction, all loci including *D95* were in Hardy-Weinberg equilibrium.

In the farm bred Malaysian yellow-tail gold arowana, there were five significant deviations from Hardy-Weinberg equilibrium (Table 4.3). Highly significant ($P < 0.01$) heterozygosity deficiencies were at loci *D13*, *D31*, *D32* and *D95* while locus *D27* showed a weaker significance ($P < 0.05$) of heterozygosity deficiecy. Four departures from Hardy-Weinberg equilibrium were observed following sequential Bonferroni correction. Loci *D13*, *D15* and *D32* and *D95* showed highly significant ($P < 0.01$) heterozygosity deficiecies.

Table 4.3. P-value of the arowana strains to the Hardy-Weinberg expectation (Gua and Thompson, 1992; Raymond and Rousset, 1995)

Locus		Wild Green	Farm Green	Malaysian Red-tail Gold	Red	Wild Malaysian Yellow-tail Gold	Farm Malaysian Yellow-tail Gold	Indonesian Gold
D01	H_o	0.16667	0.85714	0.68421	0.66667	0.83333	0.87500	0.54545
	H_e	0.16667	0.79630	0.61309	0.67586	0.75758	0.63911	0.73593
	F_{IS}	-	-0.114	-0.120	-0.045	-0.111	-0.386	0.200
	P	-	0.8891	0.7097	0.7486	0.8628	0.9966	0.1517
D04	H_o	1.00000	0.84615	0.77273	0.13333	0.85714	0.75000	0.63636
	H_e	0.54545	0.63077	0.73573	0.30805	0.72527	0.75403	0.79654
	F_{IS}	-1.000	-0.361	-0.083	0.447	-0.220	-0.017	0.176
	P	1	1	0.1221	0.0370	0.3376	0.4455	0.2595
D11	H_o	-	0.92857	0.80000	0.40000	-	0.56250	0.54545
	H_e	-	0.66402	0.61839	0.33103	-	0.45766	0.61905
	F_{IS}	-	-0.420	-0.394	-0.217	-	-0.239	0.124
	P	-	0.9846	0.9928	1	-	1	0.4645
D13	H_o	-	-	0.61905	0.00000	0.00000	0.00000	0.27273
	H_e	-	-	0.70732	0.64138	0.39560	0.53427	0.58009
	F_{IS}	-	-	0.128	1.000	1.000	1.000	0.474
	P	-	-	0.3890	0.0000**	0.0769	0.0000**	0.0438

(continued)

(continued) Table 4.3. P-value of the arowana strains to the Hardy-Weinberg

D14	H_o	1.00000	1.00000	0.27273	0.33333	0.57143	0.62500	0.54545
	H_e	0.54545	0.60582	0.67865	0.55402	0.75824	0.70766	0.59307
	F_{IS}	-1.000	-0.693	0.584	0.349	0.200	0.091	0.084
	P	1	1	0.0052	0.0228	0.3393	0.3900	0.6344
D15	H_o	1.00000	0.33333	0.18750	0.00000	0.42857	0.86667	0.09091
	H_e	0.55556	0.57609	0.63105	0.49206	0.36264	0.50805	0.67100
	F_{IS}	-1.000	0.371	0.683	1.000	-0.200	-0.750	0.853
	P	1	0.4388	0.0403	0.0003**	1	1	0.0001**
D16	H_o	-	0.35714	0.40000	0.58333	0.57143	0.35714	0.36364
	H_e	-	0.36243	0.32821	0.67029	0.53846	0.36243	0.45455
	F_{IS}	-	-0.182	-0.226	1	-0.333	-0.812	0.059
	P	-	1	1	0.0001**	1	1	0.4386
D27	H_o	-	0.28571	0.18182	0.58333	0.57143	0.25000	0.27273
	H_e	-	0.57143	0.25476	0.67029	0.53846	0.59073	0.46320
	F_{IS}	-	0.453	0.156	0.135	-0.333	0.545	0.318
	P	-	0.0476	0.2240	0.1461	1	0.0070	0.1980
D31	H_o	0.33333	0.69231	0.04762	0.26667	0.00000	0.06250	0.00000
	H_e	0.43939	0.61846	0.55749	0.29655	0.39560	0.56855	0.43158
	F_{IS}	-0.111	-0.125	0.909	-0.120	1.000	0.888	1.000
	P	1	0.8120	0.0000**	1	0.0769	0.0000**	0.0093

(continued)

(continued) Table 4.3. P-value of the arowana strains to the Hardy-Weinberg

D32	H_o	0.66667	0.64286	0.13636	0.26667	0.14286	0.18750	0.09091
	H_e	0.77273	0.71164	0.29387	0.63908	0.39560	0.70968	0.17749
	F_{IS}	0.091	0.071	0.471	0.562	0.500	0.729	0.200
	P	0.5013	0.2984	0.0481	0.0060	0.0769	0.0000**	0.1517
D33	H_o	-	-	0.04545	0.28571	-	0.06250	0.18182
	H_e	-	-	0.08985	0.31481	-	0.12298	0.17316
	F_{IS}	-	-	-	-0.130	-	-	-0.053
	P	-	-	-	1	-	-	1
D35	H_o	-	-	0.13636	-	-	-	-
	H_e	-	-	0.17230	-	-	-	-
	F_{IS}	-	-	-0.050	-	-	-	-
	P	-	-	1	-	-	-	-
D37	H_o	0.00000	0.21429	0.50000	0.73333	1.00000	0.50000	0.63636
	H_e	0.63636	0.61640	0.76923	0.73333	0.60440	0.57863	0.79221
	F_{IS}	1.000	0.652	0.399	-0.020	-0.750	0.140	0.176
	P	0.0303	0.0003**	0.1056	0.0131	1	0.5724	0.0212
D38	H_o	0.66667	0.64286	0.77273	0.66667	0.71429	0.60000	0.81818
	H_e	0.59091	0.45238	0.84461	0.71034	0.83516	0.79540	0.75758
	F_{IS}	-0.429	-0.444	0.068	0.034	0.118	0.227	-0.084
	P	1	1	0.0185	0.5193	0.4163	0.3892	0.5669

(continued)

(continued) Table 4.3. P-value of the arowana strains to the Hardy-Weinberg

D42	H_o	1.00000	0.15385	0.00000	0.13333	0.85714	0.80000	0.63636
	H_e	0.66667	0.60923	0.13937	0.36322	0.61538	0.61839	0.72727
	F_{IS}	-0.579	0.724	1.000	0.569	-0.440	-0.400	0.091
	P	1	0.0338*	0.0244	0.0115	1	1	0.4411
D72	H_o	-	0.35714	0.00000	0.07143	0.14286	0.06667	0.00000
	H_e	-	0.36243	0.29733	0.45767	0.27473	0.25057	0.25974
	F_{IS}	-	0.182	1.000	0.822	-	0.650	1.000
	P	-	1	0.0003**	0.0068	-	0.1034	0.0467
D85	H_o	-	0.42857	0.50000	0.23077	0.28571	0.18750	0.54545
	H_e	-	0.72222	0.54228	0.64615	0.38462	0.23185	0.71429
	F_{IS}	-	0.368	0.021	0.613	-0.091	-0.071	0.200
	P	-	0.0686	0.0006*	0.0418	1	1	0.4174
D88	H_o	-	0.64286	0.42857	0.38462	0.28571	0.18750	0.45455
	H_e	-	0.48942	0.48200	0.32308	0.38462	0.23185	0.49784
	F_{IS}	-	-0.330	0.043	-0.200	-0.091	-0.071	0.091
	P	-	1	0.5027	1	1	1	0.5046
D92	H_o	1.00000	1.00000	0.68182	0.40000	0.42857	0.25000	0.60000
	H_e	0.78788	0.69841	0.74207	0.49195	0.59341	0.33871	0.81053
	F_{IS}	-0.304	-0.456	0.083	0.102	0.143	0.130	0.229
	P	1	1	0.5054	0.4195	0.4406	0.3059	0.3794

(continued)

(continued) Table 4.3. P-value of the arowana strains to the Hardy-Weinberg

D94	H_o	-	0.71429	0.63636	0.66667	-	0.37500	0.27273
	H_e	-	0.61640	0.47463	0.51494	-	0.59476	0.52814
	F_{IS}	-	-0.166	-0.448	-0.308	-	0.333	0.423
	P	-	0.9040	1	0.7484	-	0.3731	0.1838
D95	H_o	0.16667	0.25000	0.22727	0.13333	0.14286	0.07143	0.36364
	H_e	0.68182	0.65942	0.56448	0.51494	0.57143	0.54497	0.65801
	F_{IS}	0.706	0.582	0.570	0.723	0.714	0.862	0.459
	P	0.1515	0.0069	0.0037	0.0007*	0.0210	0.0002**	0.1011
Combined	N	6	14	22	15	7	16	11
	P	0.9657	0.4324	0.000**	0.000**	0.2307	0.0001**	0.0002**

F_{IS} = inbreeding coefficient indicating deviation from Hardy-Weinberg proportions.
P is the probability value associated with the F_{IS}. Significant HWE departure for each locus was determinced by adding * = $P<0.05$ and ** = $P<0.01$ following sequential Bonferroni correction (Rice, 1989).
P combined = combined probability over all loci in the given sample or combination of samples (Fisher, 1970). N, sample size.

Table 4.3 shows that five loci (*D13*, *D15*, *D31*, *D37* and *D72*) with significant ($P < 0.05$) heterozygosity deficiencies were detected in the Indonesian gold arowana. Among these five loci, *D15* had a significance of heterozygosity deficiency at $P < 0.01$.

4.7 Linkage Disequilibrium

Tests for genotypic disequilibrium between microsatellite loci within populations gave 59 significant P values (χ^2 test $P< 0.05$) out of 1470 possible comparison (4.01%). Global tests of linkage disequilibrium calculated from within-population data, revealed that 15 (7.5%) significant P values (χ^2 test $P < 0.05$). All P values were rendered non-significant following sequential Bonferroni correction. Thus, all the microsatellite loci tested in this study were in linkage equilibrium.

4.8 Bottleneck Analysis

Only one of the individual loci analyzed, *D38* in the red arowana showed significant ($P < 0.05$) heterozygosity excess. However, this locus did not experience any bottleneck event since the P value was not significant after Bonferroni adjustment. The results of Wilcoxon rank test in Table 4.4 showed a significant ($P < 0.05$) heterozygous excess in the wild and reared green arowana after Bonferroni adjustment. The allelic frequency distribution showed significant 'mode-shift' (lack of low frequency alleles) in the wild and reared green arowana while the other strains showed a normal L-shaped allelic frequency distribution as expected under mutation drift equilibrium. Therefore, one might conclude recent bottlenecks in the wild and reared green arowana.

Table 4.4. Observed gene diversity (H_e, Hardy-Weinberg heterozygosity), equilibrium gene diversity (H_{eq}) and excesses or deficiencies in heterozygosity under the two-phased model of mutation and the assumption of equilibrium effective population size

Locus		Wild Green	Farm Green	Malaysian Red-tail Gold	Red	Wild Malaysian Yellow-tail Gold	Farm Malaysian Yellow-tail Gold	Indonesian Gold
D01	H_e	0.167	0.772	0.613	0.639	0.758	0.639	0.675
	H_{eq}	0.347	0.681	0.452	0.671	0.688	0.583	0.490
	P	0.3020	0.1390	0.1390	0.3100	0.2680	0.3880	0.0490
D04	H_e	0.545	0.631	0.715	0.251	0.714	0.738	0.766
	H_{eq}	0.344	0.592	0.704	0.590	0.671	0.580	0.700
	P	1	0.4340	0.4460	0.0190	0.4330	0.0340	0.2590
D11	H_e	-	0.664	0.582	0.331	-	0.458	0.619
	H_{eq}	-	0.594	0.587	0.275	-	0.580	0.614
	P	-	0.3670	0.4090	0.4380	-	0.1630	0.4510
D13	H_e	-	-	0.707	0.579	0.264	0.476	0.506
	H_{eq}	-	-	0.564	0.563	0.333	0.461	0.483
	P	-	-	0.0690	0.2400	0.4670	0.4640	0.4900
D14	H_e	0.545	0.606	0.647	0.506	0.703	0.685	0.593
	H_{eq}	0.352	0.467	0.550	0.732	0.541	0.458	0.620
	P	0.0780	0.1920	0.2460	0.0220	0.0450	0.0060	0.3250

(continued)

(continued) Table 4.4. Observed gene diversity (H_e, Hardy-Weinberg heterozygosity), equilibrium gene diversity (H_{eq}) and excesses or deficiencies in heterozygosity under the two-phased model of mutation and the assumption of equilibrium effective population size

D15	H_e	0.556	0.522	0.579	0.423	0.363	0.508	0.593
	H_{eq}	0.364	0.473	0.	0.282	0.337	0.282	0.613
	P	0.0780	0.4580	0.4090	0.3090	0.5490	0.1010	0.3570
D16	H_e	-	0.304	0.328	0.519	0.440	0.304	0.385
	H_{eq}	-	0.265	0.259	0.275	0.326	0.272	0.492
	P	-	0.4500	4110	0.0230	0.3830	4640	0.2330
D27	H_e	-	0.513	0.215	0.670	0.440	0.540	0.394
	H_{eq}	-	0.594	0.560	0.604	0.334	0.660	0.490
	P	-	0.2120	0.0240	0.3490	0.3740	0.1150	0.2900
D31	H_e	0.303	0.618	0.512	0.239	0.264	0.542	0.337
	H_{eq}	0.340	0.478	0.444	0.276	0.326	0.451	0.303
	P	0.6710	0.1390	0.4210	0.5920	0.4820	0.3550	0.5060
D32	H_e	0.727	0.690	0.255	0.598	0.275	0.675	0.091
	H_{eq}	0.558	0.467	0.639	0.580	0.536	0.662	0.294
	P	0.0070	0.0030	0.0070	0.4820	0.0800	0.4860	0.2230
D33	H_e	-	-	0.045	0.254	-	0.063	0.173
	H_{eq}	-	-	0.261	0.286	-	0.272	0.291
	P	-	-	0.1700	0.4950	-	0.1840	0.3870

(continued)

(continued) Table 4.4. Observed gene diversity (H_e, Hardy-Weinberg heterozygosity), equilibrium gene diversity (H_{eq}) and excesses or deficiencies in heterozygosity under the two-phased model of mutation and the assumption of equilibrium effective population size

D35	H_e	-	-	0.130	-	-	-	-
	H_{eq}	-	-	0.247	-	-	-	-
	P	-	-	0.3880	-	-	-	-
D37	H_e	0.485	0.601	0.750	0.720	0.604	0.579	0.766
	H_{eq}	0.348	0.590	0.643	0.588	0.545	0.458	0.610
	P	0.3400	0.4740	0.1370	0.0850	0.4370	0.2100	0.0260
D38	H_e	0.485	0.542	0.828	0.690	0.802	0.770	0.758
	H_{eq}	0.352	0.278	0.702	0.460	0.752	0.672	0.613
	P	0.3420	0.2410	0.0050	0.0010	0.4370	0.1180	0.0340
D42	H_e	0.667	0.542	0.093	0.303	0.615	0.579	0.697
	H_{eq}	0.556	0.442	0.248	0.582	0.539	0.455	0.486
	P	0.2550	0.4260	0.3180	0.0430	0.3060	0.2270	0.0100
D72	H_e	-	0.304	0.251	0.389	0.143	0.186	0.173
	H_{eq}	-	0.266	0.256	0.273	0.325	0.267	0.297
	P	-	0.4440	0.5090	0.3440	0.3030	0.4470	0.3660
D85	H_e	-	0.669	0.511	0.582	0.264	0.175	0.675
	H_{eq}	-	0.472	0.556	0.470	0.339	0.269	0.484
	P	-	0.0360	0.2920	0.2350	0.4470	4210	0.0410

(continued)

(continued) Table 4.4. Observed gene diversity (H_e, Hardy-Weinberg heterozygosity), equilibrium gene diversity (H_{eq}) and excesses or deficiencies in heterozygosity under the two-phased model of mutation and the assumption of equilibrium effective population size

D88	H_e	-	0.489	0.447	0.323	0.264	0.175	0.498
	H_{eq}	-	0.462	0.643	0.296	0.328	0.270	0.495
	P	-	0.4770	0.0630	0.4950	0.4790	0.4130	0.4400
D92	H_e	0.788	0.698	0.742	0.444	0.495	0.286	0.768
	H_{eq}	0.689	0.673	0.699	0.590	0.666	0.582	0.770
	P	0.0730	0.4910	0.3700	0.1340	0.1050	0.0270	0.4200
D94	H_e	-	0.616	0.444	0.515	-	0.556	0.463
	H_{eq}	-	0.596	0.253	0.269	-	0.460	0.619
	P	-	0.5110	0.2360	0.0480	-	0.3000	0.1170
D95	H_e	0.530	0.583	0.521	0.469	0.473	0.500	0.658
	H_{eq}	0.344	0.473	0.439	0.588	0.537	0.462	0.615
	P	0.1860	0.2960	0.3740	0.1670	0.2860	0.5230	0.4340
P		0.00464* excess	0.00010* excess	0.43244 none	0.36425 none	0.40879 none	0.24900 none	0.32383 none

Significant P values are indicated by *($P < 0.05$) after Bonferroni adjustment

4.9 Genetic Differentiation Among Strains

The distribution of shared alleles (Table 4.5) indicated that the Malaysian-red tail gold arowana shared the highest proportion of alleles with the other strains, followed by the farm bred Malaysian yellow-tail gold arowana. The wild green arowana was the most distinctive strain since it shared the least proportion of alleles with the other strains.

In this study, F_{ST} values ranged from a low of 0.06131 between the wild and farm bred Malaysian yellow-tail gold arowana to a high of 0.43101 between the wild green arowana and the red arowana. All pair-wise F_{ST} values ($P < 0.05$) except the F_{ST} value between the wild and reared Malaysian yellow-tail gold arowana were significantly different from zero. This revealed relatively strong genetic differentiation among all strains except the Malaysian yellow-tail gold arowana. The wild green arowana was the most highly differentiated strain (average $F_{ST} = 0.3285$), followed by the red arowana (average $F_{ST} = 0.332245$) and the wild Malaysian yellow-tail gold arowana (average $F_{ST} = 0.26228$). Therefore, the wild green arowana was the most distant from the other strains while the Malaysian red-tail gold arowana was the least distant.

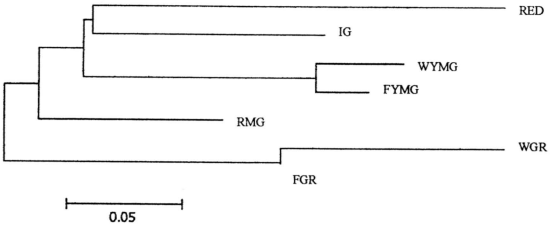
The neighbor-joining tree in Fig. 4.23 displays the relationships among the seven strains of arowana according to the microsatellite data. The tree topology indicates that the seven strains of arowana were monophyletic and there were five distinct lineages. The wild and farm bred green arowana formed an outgroup and showed a basal position among the seven strains of arowana.

Table 4.5: Pairwise comparisons of shared microsatellite alleles (above diagonal) and F_{ST} values (below diagonal) between 7 strains of arowana

Strains	Wild Green	Farm Green	Malaysian Red-tail Gold	Red	Wild Malaysian Yellow-tail Gold	Farm Malaysian Yellow-tail Gold	Indonesian Gold
Wild Green		36	31	22	26	27	27
Farm Green	0.09595*		49	36	38	42	43
Malaysian Red-tail Gold	0.31631*	0.20986*		45	45	51	54
Red	0.43101*	0.31125*	0.28801*		34	41	44
Wild Malaysian Yellow-tail Gold	0.42821*	0.31750*	0.20576*	0.32358*		52	43
Farm Malaysian Yellow-tail Gold	0.37934*	0.28809*	0.21242*	0.30258*	0.06131		49
Indonesian Gold	0.32039*	0.26787*	0.22033*	0.27827*	0.23730*	0.22931*	

An asterisk indicates a significant genetic distance ($P < 0.05$).

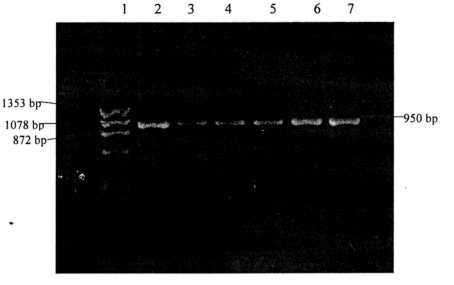
Fig. 4.24 Neighbor-joining (NJ) tree of arowana based on microsatellite data



WGR: Wild green
FGR: Farm greem
WYMG: Wild Malaysian yellow-tail gold
FYMG: Farm Malaysian yellow-tail gold
RMG: Malaysian red-tail gold
IG: Indonesian gold

4.10 MtDNA Sequence Variability

Fig. 4.25 MtDNA fragment of *ATPase6* and *ATPase8*



Lane 1: Roche DNA molecular weight marker
Lane 2 to 7: Mt DNA fragment

PCR products of approximately 950 bp of fragment size were amplified (Fig. 4.25). A total of 842 bp sequences containing the *ATPsae6* and *ATPase8* subunits were determined and 10 haplotypes were identified. The haplotypic variabilities of the 10 samples are listed in Appendix 5. The nucleotide composition (relative values) of the *ATPase6* and *ATPase8* sequences in the arowana were: A: 28.03% to 28.27%, T: 25.36% to 25.77%, C: 34.20% to 34.98% and G: 11.46% to 12.00%. Cytosine was the most common nucleotide. There was a bias to cytosine and adenine (62.23% to 63.13%). The sequences of these haplotypes were submitted to GenBank. The Genbank accession numbers for each of the haplotypes are listed in Appendix.6.

Table 4.6 shows that there were 35 (4.16%) variable sites (24 transitions and 11 transversions). Most transversions were between guanine and cytosine (45.5%), whereas adenine-cytosine, adenine-thymine and guanine-thymine transversions accounted for 27.3%, 18.2% and 9.09% respectively. Over half (58.3%) of the transitions were between cytosine and thymine. The ratio of transition (TS) and transversion (TV) was 2.18: 1. Seven site replacements were potentially phylogenetically informative and most of these substitutions occurred in the red arowana. Table 4.7 shows that slightly more than half (51.4%) of the variation was located in the third codon position. Seventeen amino acid positions were variable. Four amino acid positions were informative, with two defining the red arowana, one defining the Malaysian yellow-tail gold arowana and another one definingthe Indonesian gold arowana.

4.11 Strains Differentiation

The analysis of molecular variance (AMOVA) in Table 4.8 using molecular diversity among haplotypes showed significant genetic differentiation among the colour strains (ϕ_{ST}= 0.429, $p < 0.01$). This suggests that 42.9% of the total genetic variance observed was due to differences among strains.

Pairwise sequence divergence values (Table 4.9) ranged from an intra strain average of 0.2% to an inter strain average of 2.6%. Intra strain divergence was low, especially among the three strains of gold arowana. It ranged from 0.2% (the Malaysian yellow-tail gold and Indonesian gold) to 0.5% (the Malaysian red-tail gold). Higher divergence was observed in the red and green arowana, showing 2.0% and 1.4% divergence respectively. Sequence divergence in the pairwise comparisons

among the gold arowana ranged from 0.4% (between the Malaysian red-tail gold arowana and the Malaysian yellow-tail gold arowana) to 0.6% (between the Malaysian red-tail gold arowana and the Indonesian gold arowana). The divergence between the gold and red arowana ranged from 2.2% (between the Malaysian red-tail gold and red arowana) to 2.45% (between the Indonesian gold and red arowana). Divergence between the gold and green arowana ranged from 1.18% (between the Indonesian gold and green arowana) to 1.3% (between the Malaysian yellow-tail gold and green arowana).

The neighbor-joining (NJ) tree topology (Fig. 4.26) was supported with 39% to 98% bootstrap values. The NJ tree revealed the monophyly of the arowana, with two different lineages in it. The gold arowana formed a sister clade with the green. However, the relationships amongthe three strains of gold arowana were not resolved confidently since the bootstrap values of their relationship were quite low (39% to 54%). The red arowana was the most distant group and had a basal position within the five colour strains.

Table 4.6: Variable sites for *ATPase8* and *ATPase6*. Dots indicate identity to the sequence of GR15

	4	8	1	1	1	1	1	2	2	2	2	3	3	3	3	3	3	3	4	5	5	5	5	5	6	6	7	7	7	7	8	8	8	8
	2	4	0	1	2	2	4	0	3	3	4	0	1	2	2	6	6	7	5	0	1	1	3	3	6	7	1	3	4	5	6	0	2	2
			2	1	1	3	1	0	0	9	0	5	0	7	9	6	8	4	4	1	3	4	2	4	8	8	1	2	7	0	7	0	4	5	7
GR15	A	T	T	A	G	T	A	C	C	G	T	A	T	C	A	A	A	C	T	C	C	G	C	C	A	C	T	T	G	C	A	C	T	C	A
GR19	G	C	.	G	A	C	.	T	.	A	.	.	.	.	.	G	.	.	.	.	.	.	.	.	G	.	A	.	C	.	.	.	.	.	.
RMG7	.	.	.	.	.	.	.	.	.	.	.	C	.	.	G	G	.	T	.	.	.	.	.	.	.	.	A	.	C	A	.	.	.	.	.
RMG18	.	.	.	.	.	.	.	.	.	.	.	.	.	T	G	.	.	T	.	.	.	.	.	.	.	.	A	.	C	A	.	.	.	.	.
R2	.	.	C	.	.	.	C	.	T	.	C	.	.	.	G	.	T	.	.	T	G	A	T	G	.	G	A	G	.	A	G	.	.	.	G
R10	G	.	.	.	.	.	.	.	T	.	.	.	.	.	G	.	T	.	.	T	G	.	.	.	.	.	A	.	C	A	G	G	C	.	.
YMG4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	T	.	.	.	.	.	.	.	.	A	.	C	A	.	.	.	T	.
YMG6	.	.	.	.	.	.	.	.	.	.	.	C	.	.	G	G	.	T	.	.	.	.	.	.	.	.	A	.	C	A	.	.	.	T	.
IG1	.	.	.	.	.	.	.	.	.	.	.	.	C	.	G	G	.	T	C	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.
IG6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	T	C	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.

Table 4.7: *ATPase* base variability by codon position

	First position	Second position	Third position	Total
ATPase 8				
Total base pair sites	56	56	56	168
Variable base pair sites	1	0	6	7
Percentage variable sites	1.5%	0%	10.7%	12.2%
Informative base pair sites	0	0	0	0
Percentage informative sites	0%	0%	0%	0%
ATPase 6				
Total base pair sites	228	228	228	684
Variable base pair sites	12	4	12	28
Percentage variable sites	5.3%	1.8%	5.3%	12.4%
Informative base pair sites	3	1	3	7
Percentage informative sites	1.3%	0.4%	1.3%	3%

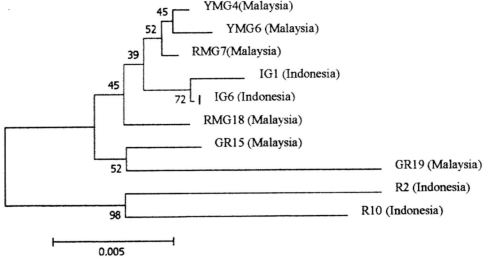
Table 4.8: Results of analysis of molecular variance (AMOVA)

Source of variation	d.f.	Sum of square	Variance components	% of total variance
Among strains	4	28.00	2.10	42.86
Within strains	5	14.00	2.80	57.14
Total	9	42.00	4.90	
Fixation Index	$\phi_{st} = 0.429$	$P < 0.01$		

Table 4.9: Pairwise mtDNA sequence divergence

Haplotypes	Green 15	Green 19	Malaysian Red-tail Gold 7	Malaysian Red-tail Gold 18	Red 2	Red 10	Malaysian Yellow-tail Gold 4	Malaysian Yellow-tail Gold 6	Indonesian Gold 1	Indonesian Gold 6
Green 15										
Green 19	0.013									
Malaysian Red-tail Gold 7	0.008	0.014								
Malaysian Red-tail Gold 18	0.008	0.017	0.005							
Red 2	0.020	0.031	0.021	0.021						
Red 10	0.021	0.027	0.020	0.020	0.019					
Malaysian Yellow-tail Gold 4	0.008	0.014	0.002	0.005	0.021	0.020				
Malaysian Yellow-tail Gold 6	0.011	0.017	0.002	0.007	0.024	0.023	0.002			
Indonesian Gold 1	0.010	0.015	0.006	0.008	0.025	0.024	0.006	0.006		
Indonesian Gold 6	0.007	0.013	0.004	0.006	0.021	0.021	0.004	0.006	0.002	

Fig. 4.26 Neighbor-joining (NJ) tree of mtDNA *ATPase* haplotypes of the arowana



GR: Green
RMG: Malaysian red-tail gold
YMG: Malaysian yellow-tail gold
IG: Indonesian gold
R: Red